

Listing of claims:

1. (Currently amended) A method for electromanipulation of at least one cell or cell-like structure having cell-like membranes, comprising the following consecutive steps:

(a) at least one cell or cell-like structure is transported from one or more sample containers located on a chip through at least one microchannel located on said chip into a chamber located on said chip, wherein said chamber contains at least one microelectrode connected to a voltage generator, and wherein said microchannel provides a fluid contact between the sample containers,

(b) either said at least one cell or cell-like structure is placed or aligned close to said at least one electrode, or said at least one microelectrode is placed or aligned close to said at least one cell or cell-like structure in said chamber,

(c) an electrical field is applied and focused on said at least one cell or cell-like structures, said electrical field being of a strength sufficient to obtain pore-formation in said at least one cell or cell-like structure or sufficient to obtain fusion of said at least one cell or cell-like structure with another cell or cell-like structures ~~present~~ in said chamber.

2. (Original) The method of Claim 1, wherein step (a) wherein the transport of said at least one cell or cell-like structures inside said microchannels is performed by optical trapping in combination with device translation.

3. (Original) The method of Claim 1, wherein at least one agent is delivered through said pore formed in step (c) into said at least one cell or cell-like structure.

4. (Original) The method of Claim 3, wherein said at least one agent is delivered

through said pore and into said cell or cell-like structure followed by delivery of at least one other agent through said pore and into said cell or cell-like structure cell structure, wherein the deliveries of the different agents are performed in a sequential manner.

5. (Original) The method of Claim 3, wherein said at least one agent is delivered through said pore and into said cell or cell-like structure followed by delivery of at least one other agent through said pore and into said cell or cell-like structure cell structure, wherein the deliveries of the different agents are performed in a parallel manner.

6. (Original) The method of Claim 4, wherein the deliveries of the different agents are performed in a combinatorial manner.

7. (Original) The method of Claim 5, wherein the deliveries of the different agents are performed in a combinatorial manner.

8. (Original) The method of Claim 4, wherein said agent is selected from the group consisting of pharmaceutically active compounds, electrolytes, substances that activates receptors on the cell plasma membrane, agents that affects intracellular chemistry, agents that affects cellular physics, genes, gene analogs, RNA, RNA analogs, DNA, DNA analogs, colloidal particles, receptors, receptor ligands, receptor antagonists, receptor blockers, enzymes, enzyme substrates, enzyme inhibitors, enzyme modulators, proteins, protein analogs, amino acids, amino acid analogs, peptides, peptide analogs, metabolites, metabolite analogs, oligonucleotides,. oligonucleotide analogs, antigens, antigen analogs, haptens, hapten analogs, antibodies, antibody analogs, organelles, organelle analogs, cell nuclei, bacteria, viruses, gametes, inorganic ions, metal ions, metal clusters, polymers, and any combinations thereof.

9. (Original) The method of Claim 5, wherein said agent is selected from the group consisting of pharmaceutically active compounds, electrolytes, substances that activates receptors on the cell plasma membrane, agents that affects intracellular chemistry, agents that affects cellular physics, genes, gene analogs, RNA, RNA analogs, DNA, DNA analogs, colloidal particles, receptors, receptor ligands, receptor antagonists, receptor blockers, enzymes, enzyme substrates, enzyme inhibitors, enzyme modulators, proteins, protein analogs, amino acids, amino acid analogs, peptides, peptide analogs, metabolites, metabolite analogs, oligonucleotides, oligonucleotide analogs, antigens, antigen analogs, haptens, hapten analogs, antibodies, antibody analogs, organelles, organelle analogs, cell nuclei, bacteria, viruses, gametes, inorganic ions, metal ions, metal clusters, polymers, and any combinations thereof.

10. (Original) The method of Claim 1 wherein step (a) wherein the transport of said at least one cell or cell-like structures inside said microchannels is performed by electrophoresis.

11. (Original) The method of Claim 1, wherein the transport of said at least one cell or cell-like structures inside said microchannels is performed by electroosmotic flows.

12. (Original) The method of Claim 1, wherein the transport of said at least one cell or cell-like structures inside said microchannels is performed by dielectrophoresis.

13. (Original) The method of Claim 1, wherein the transport of said at least one cell or cell-like structures inside said microchannels is performed by gravitational liquid flows.

14. (Original) The method of Claim 1, wherein the transport of said at least one cell or cell-like structures inside said microchannels is performed by pressurized liquid flows.

15. (Original) The method of Claim 1, wherein steps (a) - (c) are repeated until a desired number of cell or cell-like structures have been fused together.

16. (Original) The method of Claim 1 comprising a further step (d) performed after step (c), wherein electroporated or fused said at least one cell or cell-like structure is transported to a storage container in fluid contact with said chamber.

17. (Original) The method of Claim 1, wherein said at least one microelectrode is positioned in step (b) by means of a microscope, a micropositioner or a stereotactic device.

18. (Original) The method of Claim 1, wherein said microelectrode being sufficiently small to enable selective fusion of said cells or cell-like structures.

19. (Currently amended) The method of Claim 1, wherein two microelectrodes are used in step (c) and said two microelectrodes are sufficiently small to enable selective fusion of said cells or cell-like structures.

20. (Currently amended) The method of Claim 1, wherein one microelectrode is movably mounted on a microchip ~~is used in step~~, said microelectrode being sufficiently small to enable selective fusion of said cells or cell-like structures.

21. (Original) The method of Claim 20, wherein said microchip is the same chip on which the container, the microchannel and the chamber are located.

22. (Currently amended) The method of Claim 1, wherein several microelectrodes individually movably mounted on a microchip ~~is used in step~~, said microelectrodes being sufficiently small to enable selective fusion of said cells or cell-like structures.

23. (Original) The method of Claim 1, wherein said microchip is the same chip on which the container, the microchannel and the chamber are located.

24. (Currently amended) The method of Claim 1, wherein at least one of the at least one electrodes is a hollow microelectrode ~~is used~~.

25. (Original) The method of Claim 24, wherein said hollow microelectrode is located on the same chip on which the container, the microchannel and the chamber are located.

26. (Original) The method of Claim 24, wherein said hollow microelectrode is filled with an electrolyte and wherein at least one agent is delivered by electroendoosmosis, electrophoresis or Poiseuille flow through the microelectrode into the fusion container.

27. (Original) The method of Claim 25, wherein said hollow microelectrode is filled with an electrolyte and wherein at least one agent is delivered by electroendoosmosis, electrophoresis or Poiseuille flow through the microelectrode into the fusion container.

28. (Original) The method of Claim 1, wherein the outer diameter of said at least one microelectrode is 0.05-100 μm .

29. (Original) The method of Claim 1, wherein the outer diameter of said at least one electrode is 1-50 μm .

30. (Original) The method of Claim 1 wherein said at least one microelectrode provided in step (c) also is used for positioning of said at least cells or cell-like structures in step (b).

31. (Original) The method of Claim 1, wherein said cells or cell-like structures independently are selected from the group consisting of cells, liposomes, proteoliposomes, synthetic vesicles, egg cells, enucleated egg cells, sperm cells at any developmental stage and plant proteoplasts.

32. (Original) The method of Claim 1, wherein said at cell-like structures are liposomes.

33. (Original) The method of Claim 1, wherein said at least one cell or cell-like structure is a cell.

34. (Original) The method of Claim 1, wherein at least one of said cells or cell-like structures is a liposome and at least one of said cells or cell-like structures is a cell.

35. (Original) The method of Claim 1, wherein the cell or cell-like structures to be transported in step (a) is selected from a library of cells or cell-like structures in a combinatorial manner.

36. (Original) The method of Claim 1, wherein the electrical field in step (d) is applied by use of a low-voltage pulse-generator.

37. (Original) The method of Claim 1, wherein the sample containers containing the cells or cell-like structures also contains a buffer.

38. (Original) The method of Claim 1, wherein said at least one microelectrode provided in step (c) is used for electroporation of said cells or cell-like structures between steps (a) and (b).

39. (Original) The method of Claim 1, wherein at least one of said cells or cell-like structures is exposed to a dielectrophoretic field in a buffer prior to step (b).

40. (Original) The method of Claim 1, wherein at least one of said cells or cell-like structures is treated with a fusogenic or other agent that promotes close cell-cell contacts prior to step (b).

41. (Original) An apparatus for electromanipulation of at least one cell or cell-like structure having cell-like membranes, said apparatus comprising one or more sample containers for said cell or cell-like structure in fluid contact through at least one microchannel with a fusion chamber, optical trapping means for transport of individual cells or cell-like structures through said at least one microchannel into the fusion chamber, and at least one microelectrode connected to a voltage generator for providing a focused electrical field in the fusion chamber, wherein said sample container, said microchannel and said fusion chamber are placed on a chip.

42. (Original) The apparatus of Claim 41, wherein said at least one microelectrode is integrated on said chip.

43. (Original) The apparatus of Claim 41, further comprising at least one

microscope, at least one micropositioner or at least one stereotactic device for positioning of said at least one microelectrode.

44. (Original) The apparatus of Claim 41, comprising only one microelectrode.
45. (Original) The apparatus of Claim 41, having two microelectrodes.
46. (Original) The apparatus of Claim 41, wherein the microelectrode is movably mounted on a microchip.
47. (Original) The apparatus of Claim 41, having several microelectrodes individually movably mounted on a microchip.
48. (Original) The apparatus of Claim 41, wherein at least one microelectrode is hollow.
49. (Original) The apparatus of Claim 48, wherein said at least one hollow microelectrode is filled with an electrolyte.
50. (Original) The apparatus of Claim 41, wherein the outer diameter of said at least one microelectrode is 0.05-100 μm .
51. (Original) The apparatus of Claim 41, wherein the outer diameter of said at least one electrode is 1-50 μm .
52. (Original) The apparatus of Claim 41, wherein said voltage generator is a low-voltage pulse-generator.

53. (Original) The apparatus of Claim 41, wherein the sample containers contains a buffer.

54. (Original) Method for *in vitro* fertilization comprising using the method according to Claim 1.

55. (Currently amended) A method for *in vitro* fertilization comprising using the apparatus according to Claim ~~37~~ 41.

56. (Original) A method for cloning using the method according to Claim 1.

57. (Original) A method for cloning using the apparatus according to Claim 41.

58. (Original) A method for cell transfection using the method according to Claim 1.

59. (Original) A method for cell transfection using the apparatus according to Claim 41.

60. (Original) A method for the production of monoclonal antibodies using the method according to Claim 1.

61. (Original) A method for the production of monoclonal antibodies using the apparatus according to Claim 41.

62. (Original) A biosensor prepared according to the method of Claim 1.
63. (Original) A biosensor comprising the apparatus of Claim 41.
64. (Original) A method for the preparation of a hybridoma using the method according to Claim 1.
65. (Original) A method for the preparation of a hybridoma using the apparatus according to Claim 41.
66. (Original) A method for the manipulation of a composition of a cellular membrane comprising using the method according to Claim 1.
67. (Original) A method for the manipulation of a composition of a cellular membrane comprising using the apparatus according to Claim 41.
68. (Original) A method for the delivery of a well-defined volume of a substance to a cell comprising using the method according to Claim 1.
69. (Original) A method for the delivery of a well-defined volume of a substance to a cell comprising using the apparatus according to Claim 41.
70. (Original) A method for the delivery of a pharmaceutically active substance to a cell using the method according to Claim 1.

U.S.S.N.: 09/996,559

Orwar, et al.

Filed: November 30, 2001

Response to Office Action mailed March 22, 2004

Page 12 of 16

71. (Original) A method for the delivery of a pharmaceutically active substance to a cell using the apparatus according to Claim 41.

72. (Original) A method for conducting stem cell research comprising using the method according to Claim 1.

73. (Original) A method for conducting stem cell research using the apparatus according to Claim 41.